CLAIMS

We claim:

1. A device for determining presence and/or amount of an analyte in a fluid sample comprising:

a mobilization zone comprising a mobile or mobilizable detectable tracer molecule;

a sample application area;

a primary capture area comprising a first immobilized binding partner having a binding affinity for the analyte and a binding affinity for the detectable tracer molecule; and

a secondary capture area comprising a second immobilized binding partner having a binding affinity for the analyte and a binding affinity for the detectable tracer molecule,

wherein the sample application area, primary capture area and secondary capture area are in fluid continuous contact, and the first immobilized binding partner has an equal or a lower apparent affinity for the analyte than it has for the detectable tracer molecule.

2. The device of claim 1, wherein the detectable tracer molecule is associated with the device in such a way that, during operation of the device, it contacts the primary capture area after a sample contacts the primary capture area.

3. The device of claim 1, wherein, during operation of the device, the detectable tracer molecule migrates through the device at a rate slower than a rate at which the analyte in a sample migrates through the device.

4. The device of claim 3, wherein slower migration of the tracer molecule is caused by a molecular weight of the tracer molecule.

5. The device of claim 3, wherein slower migration of the tracer molecule is caused by a physical or temporal placement of the tracer molecule on the device.

6. The device of claim 5, wherein the tracer molecule is placed on the device after a sample is placed on the device.

7. The device of claim 1 further comprising at least one filter pad in a path of flow of the fluid.

8. The device of claim 5, wherein the filter pad is pretreated with at least one reagent to enhance the sensitivity of the assay device.

9. The device of claim 6 wherein the at least one reagent is selected from the group consisting of buffers, detergents, and anticoagulants.

10. The device of claim 1 wherein the first and second immobilized binding partners are selected from the group consisting of antibodies, antigens and haptens.

11. The device of claim 1 wherein the first and second binding agents for the analyte are identical.

12. The device of claim 1 wherein the first and second binding agents are each anti-analyte antibodies.

13. The device of claim 1, wherein the tracer molecule comprises an analyte molecule or an analyte analog molecule.

14. The device of claim 1, wherein the tracer molecule comprises a visually detectable label.

15. The device of claim 1, wherein the analyte is selected from the group consisting of antigens of infectious diseases, antibodies to antigens of infections diseases, hormones, growth factors, therapeutic drugs, drugs of abuse and products of the metabolism of drugs of abuse, and haptens.

16. The device of claim 15, wherein the analyte antibodies are selected from the group consisting of antibodies to HIV, antibodies to HTLV, antibodies to *Helicobacter pylori*, antibodies to hepatitis, antibodies to measles, antibodies to mumps, and antibodies to rubella.

17. The device of claim 15, wherein the therapeutic drugs and drugs of abuse or products of the metabolism of drugs of abuse are selected from the group consisting of tetrahydrocannabinol, nicotine, cotinine, ethanol, theophylline, phenytoin, acetaminophen, lithium, diazepam, nortryptyline, secobarbital, and phenobarbitol, methamphetamine and fragments, mimetics, analogs or derivatives thereof.

18. The device of claim 17, wherein the analyte is a product of metabolism of a drug of abuse, and the product of metabolism comprises cotinine.

19. The device of claim 15, wherein the hormones are selected from the group consisting of testosterone, estradiol, estriol, 17-hydroxyprogesterone, progesterone, thyroxine, thyroid stimulating hormone, follicle stimulating hormone, and luteinizing hormone, and fragments, mimetics, analogs or derivatives thereof.

20. The device of claim 1, wherein the quantity of the second specific binding partner in the secondary capture area is such that the quantity of tracer molecule binding to the secondary capture area, and by correlation the amount of the analyte in a tested sample, is indicated by the intensity of detection signal of the tracer molecule in the secondary capture area.

21. The device of claim 1 wherein the area of the secondary specific binding partner immobilized on the chromatographic medium is divided into at least two discrete and non-overlapping bands, with the quantity of the second specific binding partner in each band being such that the quantity of tracer molecule binding to the secondary capture area, and by correlation the amount of the analyte in a tested sample, is indicated by the number of bands to which the tracer molecule binds.

22. A device for determining presence and/or amount of an analyte in a fluid sample comprising:

a mobilization zone comprising a mobile or mobilizable detectable tracer molecule;

a sample application area;

a primary capture area comprising a first immobilized binding partner having a binding affinity for the analyte and a binding affinity for the detectable tracer molecule; and

a secondary capture area comprising a second immobilized binding partner having a binding affinity for the analyte and a binding affinity for the detectable tracer molecule,

wherein, during operation of the device, the detectable tracer molecule contacts the primary capture area after the sample contacts the primary capture area.

23. A method for detecting and/or quantitating an analyte in a fluid sample, comprising:

applying a liquid sample to a substrate along which the samples migrates sequentially to a primary capture area and a secondary capture area, wherein the primary capture area binds the analyte with an equal or a lower apparent affinity than it binds a detectable tracer molecule; and the secondary capture area binds the detectable tracer molecule with high affinity; and

reading a detectable signal from bound detectable tracer molecule in the secondary capture area, wherein the detectable signal indicates the presence of analyte in the sample.

24. The method of claim 23, further comprising:

applying a detectable tracer molecule to the substrate.

25. The method of claim 24, wherein the detectable tracer molecule is applied to the substrate before the sample.

26. The method of claim 24, wherein the detectable tracer molecule is applied to the substrate after the sample.

27. The method of claim 24, wherein the detectable tracer molecule and the sample are applied simultaneously.

28. The method of claim 23, wherein the detectable signal has an intensity, and the intensity of the signal correlates with the amount of analyte in the sample.

29. The method of claim 23, wherein the first and second binding partners are immobilized on the substrate.

30. The method of claim 23, using the device of claim 1.

31. A method for detecting and/or quantitating an analyte in a fluid sample, comprising:

contacting the fluid sample with the device of claim 1.

32. The method of claim 31, comprising:

applying the sample to the sample application area of the device;

allowing the sample to migrate along the device; and

allowing the detectable tracer molecule to migrate along the device, wherein a detectable signal from bound detectable tracer molecule in the secondary capture area indicates the presence of the analyte in the sample.

33. The method of claim 31, wherein the mobile detectable analyte analog is applied to the device no earlier than the sample is applied to the device.

34. The method of claim 33, wherein the analyte-tracer conjugate is mixed with the sample prior to application to the sample application area.

35. The method of claim 31, further comprising quantifying the amount of analyte in the sample, wherein the amount of analyte in the sample is proportional to the signal in the second capture area.

36. The method of claim 31, wherein the sample migrates along the test strip device by capillary action.

37. The method of claim 31, wherein the analyte has a molecular weight of about 100 – 1,000 Daltons.

38. The method of claim 31, wherein the analyte has a molecular weight of greater than 1,000 Daltons.

39. The method of claim 31, wherein the sample is a fluid sample.

40. The method of claim 39, wherein the fluid sample is selected from the group consisting of urine, blood, tears, sweat and saliva.

41. The method of claim 40, wherein the fluid sample is saliva.

42. The method of claim 41, further comprising providing an oral fluid sample combined with a bile acid bile or salt in a concentration sufficient to reduce occurrence of false positives in the immunoassay.

43. The method of claim 42, wherein the bile acid or bile salt ranges in concentration from about 0.1 weight percent to about 1.0 weight percent of the oral fluid/bile salt or bile acid combination.

44. The method of claim 43, further comprising contacting a chelator of divalent cations with the oral fluid sample.

45. A test kit for the detection and/or the determination of an analyte in a sample comprising:

(a) the chromatographic assay device of claim 1; and

(b) instructions.

46. The kit of claim 45, further comprising an aliquot of analyte-tracer conjugate.